# DEVELOPMENTAL MORPHOLOGY OF ASCOMYCETES XII. *THYRONECTRIA PSEUDOTRICHIA*

by C.V. SUBRAMANIAN and D. JAYARAMA BHAT *

SUMMARY. – Developmental study on *Thyronectria pseudotrichia* and its anamorph *Stilbella cinnabarina*. Discussion about the genus *Thyronectria* perithecial centrum which is of the typical *Nectria* type.

RÉSUMÉ. - Etude du développement de *Thyronectria pseudotrichia* et de son anamorphe *Stilbella cinnabarina*. Discussion à propos du genre *Thyronectria* dont l'organogenèse périthéciale est du type *Nectria*.

MOTS-CLÉS : Ascomycètes - Organogenèse.

## INTRODUCTION

This paper is the twelfth in a series on the developmental morphology of Ascomycetes and deals with *Thyronectria pseudotrichia* (Schw. ex Berk. & Curt) Seeler. Our observations are based on a study of the fungus collected by us on dead twigs of *Ficus asperrima* Roxb. at Abby Falls, Coorg district, Karnataka State, India.

*Nectria pseudotrichia* Schw. ex Berk. & Curt. is based on sub. *Sphaeria pseudotrichia* Schw. Recognizing the stilboid nature of its conidial state. BERKELEY & BROOME (1875) transferred *Nectria pseudotrichia* to the genus *Sphaerostilbe* Tul., as *S. pseudotrichia* (Schw. ex Berk. & Curt.) Berk. & Br. Both *Nectria* and *Sphaerostilbe* have two-celled ascospores and since *Nectria pseudotrichia* has muriform ascospores, SPEGAZZINI (1881) established a new genus *Megalonectria* for this fungus which he disposed as *M. pseudotrichia* (Schw.) Speg. WOLLENWEBER (1926) did not accept Spegazzini's genus as *Pleonectria* Sacc. (SACCARDO, 1976). It was already available for nectriaceous species with muriform ascospores and, in any case, according to him possession of a stilboid conidial state alone would not warrant a separate genus for this taxon. Accordingly, WOLLENWEBER (1926) transferred *N. pseudotrichia* to

* Centre of Advanced Study in Botany, University of Madras, Madras-600 005, India.

*Pleonectria*. The genus *Pleonectria* Sacc. is a seggregate of *Thyronectria* Sacc. *Thyronectria* was established by SACCARDO (1875) for *Nectria* species with muriform ascospores, with *T. patavina* Sacc. as type. A year after establishing the genus *Thyronectria*, SACCARDO divided this genus placing the species in which perithecia seated on a stroma in a new genus *Pleonectria* Sacc. with *P. lamyi* Sacc. as type and leaving the remaining species in *Thyronectria*. In a monographic study of the genus *Thyronectria*, SEELER (1940) disposed this taxon in *Thyronectria* as *T. pseudotrichia* (Schw. ex Berk. & Curt.) Seeler as, firstly, he did not consider the necessity of seggregating some species of *Thyronectria* into the genus *Pleonectria* on the basis of stromal character as was done by SACCARDO and secondly, he did not accept SPEGAZZINI's genus *Megalonectria* agreeing with WOLLENWEBER that the stilboid nature of the conidial state is only of limited value in generic delimitation.

The conidial state of *Thyronectria pseudotrichia* is *Stilbella cinnabarina* (Mont.) Lindau. It was originally described as *Stilbum cinnabarinum* Mont. by MONTAGNE in 1837. TODE (1790) established the genus *Stilbum* for six species and none of which were cited by FRIES (1821) in his *Systema* which is the starting point work for this group of fungi. These names, however, can be taken to be validated by MÉRAT (1821). From amongst TODE's species, JUEL (1898) designated *S. valgare* Tode as the lectotype. He also showed that *S. valgare* is a basidiomycete. The remaining species, therefore, had to be classified elsewhere and LINDAU (1900) established the genus *Stibella* for these. LINDAU (1900) did not designate a type for his genus, and, apart from TODE's five species already mentioned he included in his genus the type and other species of *Botronipha* Preuss (PREUSS, 1852) so that under the Rules. *Botryonipha* should have been used by LINDAU. In other words *Stilbella* Lindau even when it was published is illegitimate under the Rules. The correct name to be used for the conidial state of *Thyronectria pseudodotrichia* will therefore depends on whether the generic name *Stilbella* Lindau is conserved or not; in case *Stibella* Lindau is not conserved, a new generic name have to be proposed for this fungus.

The occurrence of *Thyronectria pseudotrichia* in India was first reported by SUBBA RAO (1938) on shoots and pruned branches of tea plants. He referred to the fungus as *Megalonectria pseudotrichia* (Schw.) Speg. and the conidial state as *Stilbum cinnabarinum* Mont.

## METHODS

Single ascospore isolates grown on sterilized twigs of *Ficus asperrima* and kept in Roux-tubes produced mature fruiting bodies after six weeks of incubation. For studying various stages in the development of the anamorph and teleomorph, methods described earlier (SUBRAMANIAN & BHAT, 1978) were followed.

## DESCRIPTION OF THE FUNGUS

Mycelium in fresh specimen present on the surface of the substrate, sparse, white, composed of septate, hyaline, branched, hyphae 2.5-3.2 μm wide. Perithecia (Fig. 1; Plate I,a) solitary or gregarious, in clusters of 3-12 or more, mostly globose, sometimes subglobose, ostiolate, fleshy, scarlet red or bright red, sometimes vinaceous brown, seated on a small erumpent stroma, partially immersed and confluent with stromata, when dry collapsing and becoming pezizoid; globose perithecia 280-580 (460) μm diam., subglobose perithecia 300-520 (480) X 480-500 μm; outer surface of the perithecia usually scaly, with scales withering away sometimes leaving the perithecia smooth and shiny. Perithecial wall pseudoparenchymatous, 45-85 μm thick, with two regions (Fig. 31, 32; Plate II, f) : an outer and an inner; outer region 25-45 (36) μm wide, with cells in surface view spherical, thick-walled and 9-15 μm diam., and in longitudinal section elliptical to angular, thick-walled, pigmented and 9-25 μm diam.; inner region 15-20 μm wide, with cells thin-walled, flattened, narrow, compactly arranged, hyaline and 15.0-21.0 X 2.5-3.0 μm. Stroma fleshy, pseudoparenchymatous, pigmented red, arising from below the bark, with cells towards the periphery thick-walled, angular and 5.0-12.0 μm wide, and cells towards the interior thin-walled, hyaline, 5.0-9.0 μm wide. Perithecial papilla small, appearing as a dark spot at the apex of the perithecium, with unbranched thick hyphae with rounded tips. Ostiolar canal periphysate; periphyses cylindrical, slender, 15.0-18.0 X 1.8-2.2 μm.

Asci (Fig. 46, 47; Plate II, h) elliptical to clavate, short-stalked, rounded at the apex, without apical apparatus, thin-walled, 8-spored (rarely 4, 2, or 1-spored), 75-120 (95) X 8.5-25.0 (16.5) μm, formed with the intervention of croziers. Ascospores (Fig. 45, 49; Plate II, i) muriform, constricted at the septa, ellipsoidal, broadly bulging, sometimes slightly tapering terminally, hyaline to pale brown, with granular cytoplasm, with uninucleate cells, 20-42 (25.5) X 7.0-15.0 (10.5) μm, obliquely uni- or biseriate, closely overlapping in the ascus.

Liberated ascospores producing ascoconidia (Fig. 6; Plate II, j); ascoconidia 3.0-6.0 (4.2) X 2.0-3.5 μm.

Synnemata (Fig. 1; Plate I, b) erect, solitary or in groups of 2-6 and projecting from a common point, 1.5-2.2 mm high, each with a well developed stalk and an oval to globose head, resembling drum-sticks. Stalk of the synnema stiff, with slight verrucosities due to very short projecting hyphal pegs or branching hyphae, darker below, paler above (Fig. 2; Plate I, c). Head oval to globose, orange-red in fresh specimens, brown coloured in dry specimens, composed of the free ends of the hyphae of the synnema (conidiophores) and conidia (Fig. 2, 3; Plate I, d). Conidiophores divergent, branched or unbranched, terminating in phialides; phialides in groups of 4-5 or 1-3, subcylindrical to subulate, narrowed towards the apex, 10.0-17.0 μm long, 2.0-4.0 μm wide at the base (Fig. 4, 5). Conidia slimy, solitary, obovate, 1-celled, hyaline, 5.0-8.5 (6.5) X 3.0 μm, abstricted singly and successively from the tip of the phialide and accumulating in a slimy mass.

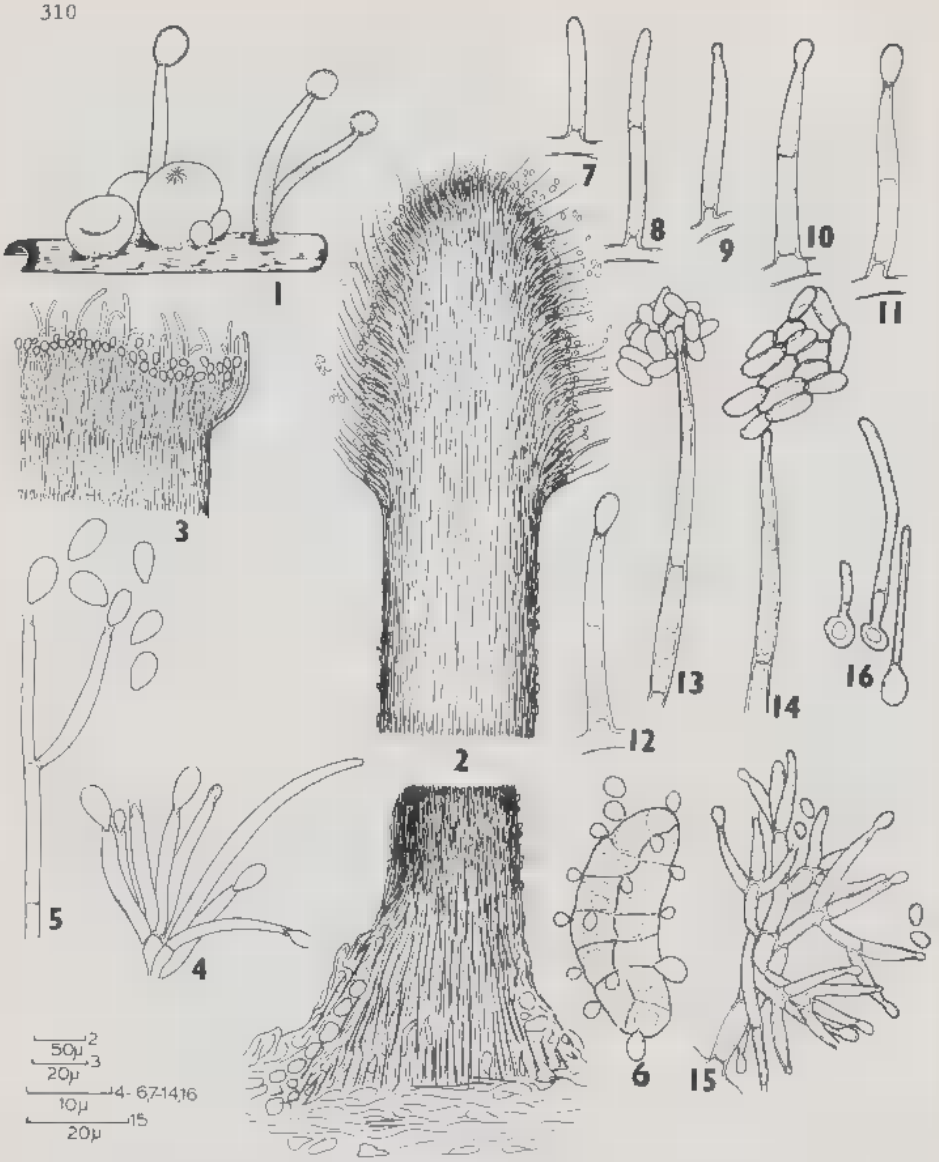



Fig. 1 - 16. – *Thyronectria pseudotrichia*
1 - perithecia and synnemata on the substrate (diagrammatic); 2 - section of a synnema; 3 - portion of synnema enlarged (head region); 4-5 - conidiophores bearing phialides and conidia (from synnema); 6 - ascospore with ascoconidia; 7 - 12 -stages in the development of phialide and first conidium; 13 -14 - phialides with slimy conidial masses at their tips; 15 - conidiophores with phialides and conidia (from culture); 16 - germinating conidia.

Fig 1 - 16 . – *Thyronectria pseudotrichia*
1 - Périthèce et synnema sur le substrat naturel (schéma); 2 - coupe dans un synnema; 3 - région apicale d'un synnema (agrandie); 4 - 5 - conidiophores portant des phialides et des conidies; 6 - ascospores et ascoconidies; 7 - 12 - différentes étapes du développement de la phialide et de la première conidie; 13 - 14 - phialides portant une tête conidienne muqueuse; 15 - conidiophores avec phialides et conidies (obtenus en culture); 16 - germination de conidies.

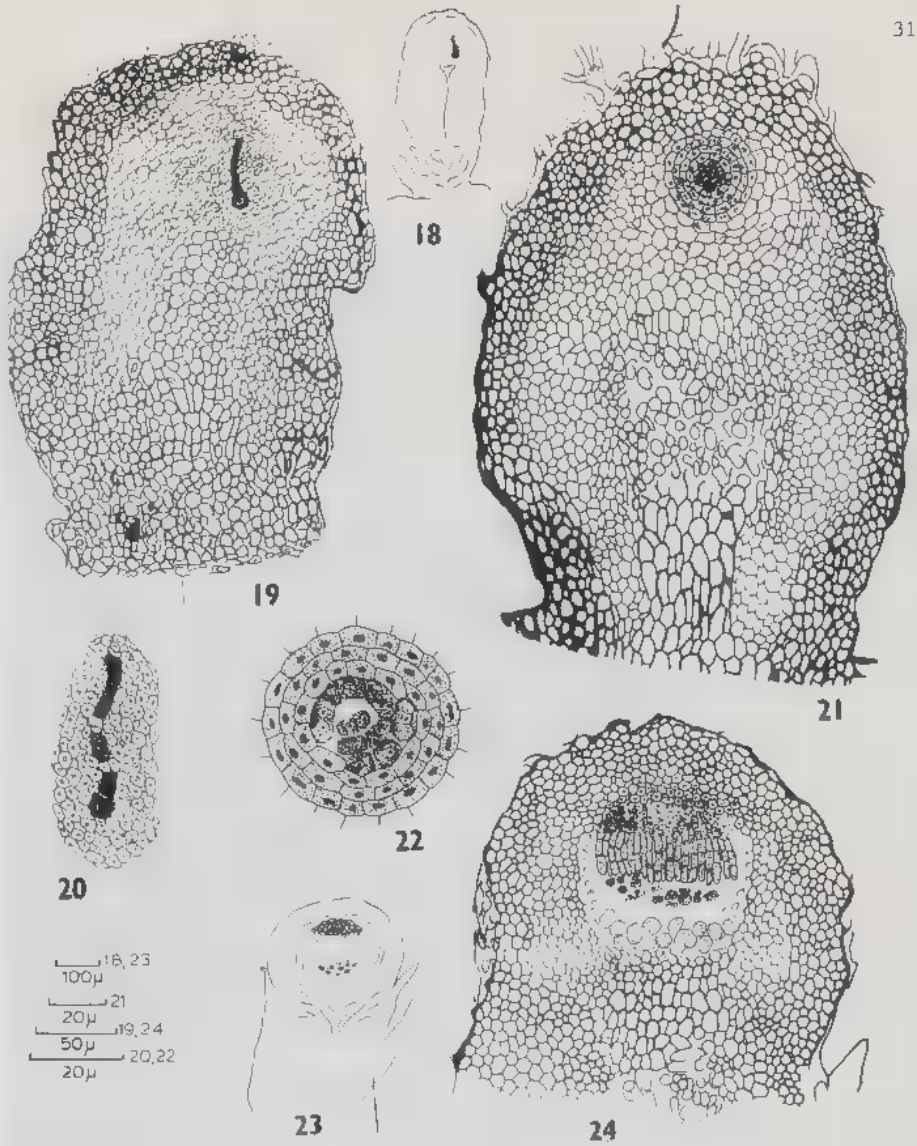
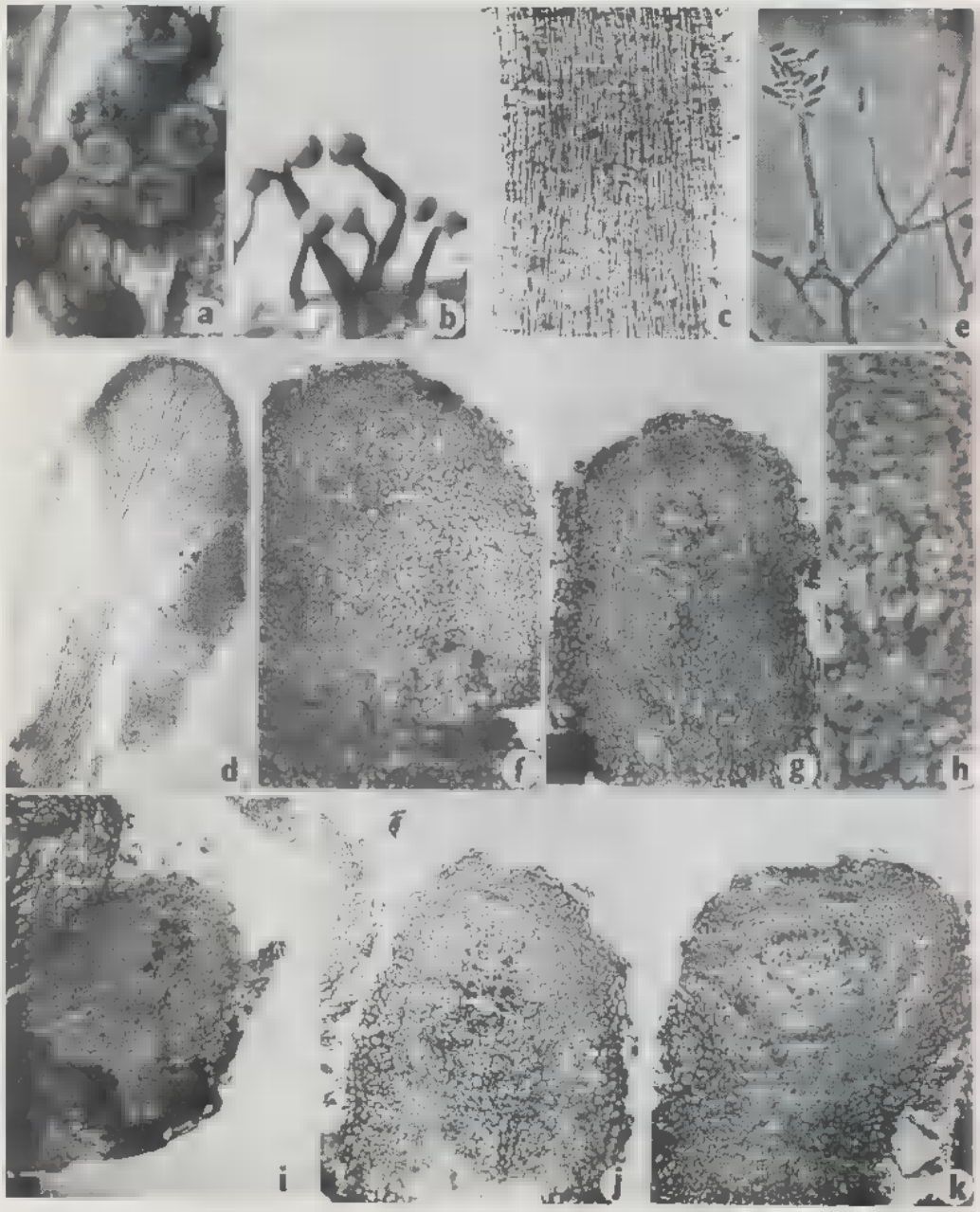



Fig. 18 - 24. – *Thyronectria pseudotrichia*
18 - 19 - longitudinal section of ■ stroma through ascogonium (portion of the stroma enlarged in 19); 20 - ascogonium with an elongate, septate trichogyne; 21 - section of stroma showing coiled ascogonium; 22 - ascogonial coil and surrounding cells enlarged; 23 - section of a stroma. Note the young perithecium protruding out of the stroma (diagrammatic); 24 - young perithecial centrum showing the development of apical paraphyses (portion of 23 enlarged).

Fig. 18 - 24. –*Thyronectria pseudotrichia*
18 - 19 - section longitudinale d'un stroma au niveau de l'ascogone; 20 - ascogone avec un trichogyne allongé et cloisonné; 21 - 22 - ascogone enroulé et cellules environnantes; 23 - coupe dans un stroma. Un jeune périthèce fait saillie à l'extérieur; 24 - développement des paraphyses apicales dans un jeune périthèce.



## CULTURAL CHARACTERS

**On agar medium :** Ascospores germinating overnight in distilled water, potato dextrose agar and malt extract agar with germ tubes arising from one or more cells in the ascospores (Fig. 50; Plate II. k). Colony on PDA yellowish-white, attaining a diameter of 4-5 cm in 10 days, with even margin, without discolouring the agar. Mycelium fast growing, floccose; aerial hyphae branched, septate, aggregating into rope-like structures. Conidiophores (Fig. 15) borne on the mycelium, short, initially mononematous, later becoming synnematous, 15.5-22.5 X 2.0-3.5 µm producing phialides terminally and laterally. Phialides subcylindrical to short-subulate, 10.0-17.0 (14.4) X 2.5-3.0 µm, with a collarette at the tip, producing conidia singly and successively (Fig. 13, 14; Plate I,e). Conidia solitary, obovate, hyaline, 1-celled, with a narrow base, 5.0-8.5 (6.5) X 3.0 µm.

**On sterilized twigs :** Mycelium on sterilized twigs of *Ficus asperima* incubated in Roux-tubes producing yellowish white colonies and covering the surface in about 10-15 days, fast growing, floccose. Conidiophores initially mononematous, later synnematous. Perithecia developing after 6 weeks on pulvinate, orange coloured stromata. Other characters as in agar culture.

## DEVELOPMENT OF THE ANAMORPH

In slide cultures, solitary conidia are produced in about 4-5 days. Phialides may be produced directly on the vegetative hyphae or it may develop in groups on phialophores, and finally attain a subcylindrical to subulate shape (Fig. 7, 8). They are broadest near the base and narrowed towards the tip. A septum cuts off each phialide from the phialophore or parent hypha.

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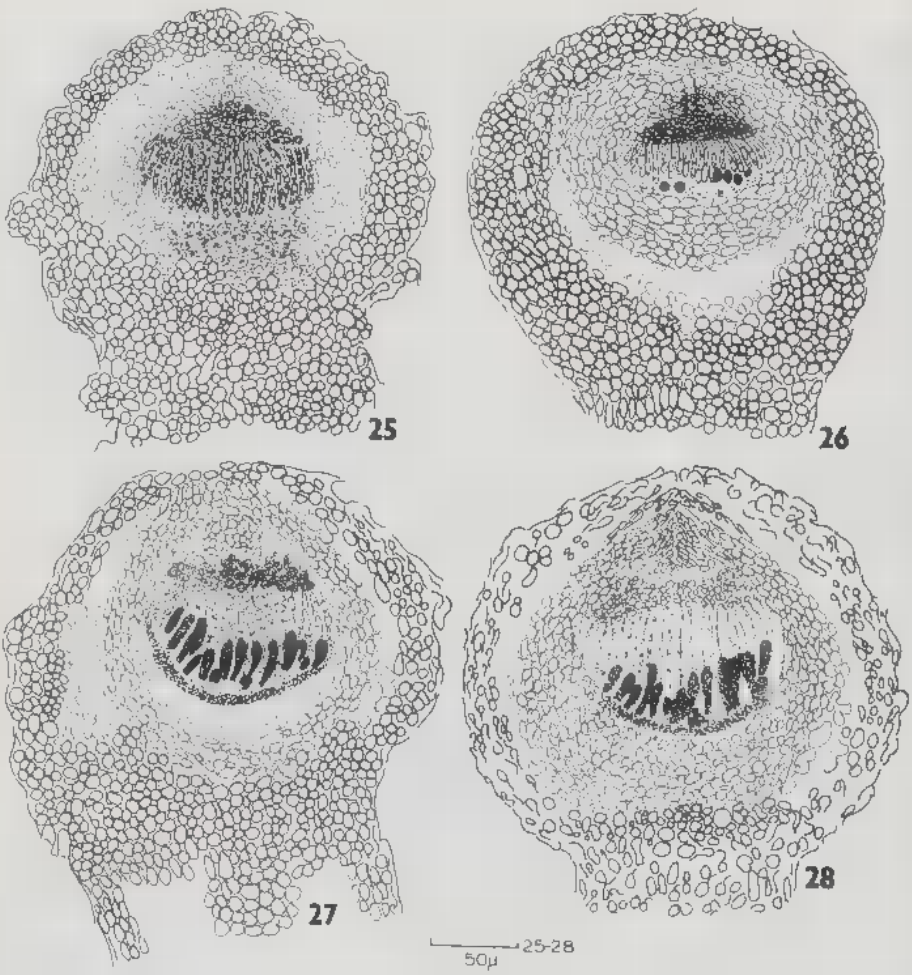
Plate I. – *Thyronectria pseudotrichia*

a - perithecia (under stereomicroscope) x23; b - synnemata x23; c - part of stalk of a synnema x485; d - section through apical part of a synnema x485; e - conidiophore with phialides and conidia x485; f - section throut part of a pseudoparenchymatous stroma x 161; g - h - section of a stroma showing an ascogonium embedded in the stroma (h, portion of g, enlarged to show the ascogonium) : g x161, h x485; i - k - stages in development of the perithecial centrum x161.

Planche I. – *Thyronectria pseudotrichia*

a - périthèce x 23; b - synnema ■ 23; c - partie du pied d'un synnema ■ 485; d - section dans la partie apicale d'un synnema x 485; ■ - conidiophore avec phialides et conidies ■ 485; f - section dans un stroma pseudoparenchymateux x 161; g - h - section dans un stroma montrant l'ascogone ■ 161 et x 485; i - k - développement du centre du périthèce x 161.




Figs. 25 - 28. – *Thyronectria pseudotrichia*
25 - 28 - longitudinal sections of perithecial centra showing the development of apical paraphyses and of asci.

Fig. 25 - 28. – *Thyronectria pseudotrichia*
25 - 28 - développement des paraphyses apicales et des asques dans un jeune périthèce (coupe).

Conidium ontogeny is as follows. The initial of the first conidium buds out as a small protuberance at the tip of the phialide (Fig. 9). During further development, the protuberance slightly elongates and swells to form the globose young conidium (Fig. 10). There is protoplasmic continuity between the conidium initial and the phialide during the initial stages of conidium development. In further development, a septum is laid down between the conidium and the phialide at the locus of constriction between them and, with the severance of the first conidium from the phialide a break of the wall of the phialide at the tip is seen (Fig. 11, 12). With the first conidium so detached, a second conidium initial appears in the open end of the phialide. This process is repeated so that a basipetal succession of solitary conidia are produced which form a slimy mass (Fig. 13).

## DEVELOPMENT OF THE TELEOMORPH

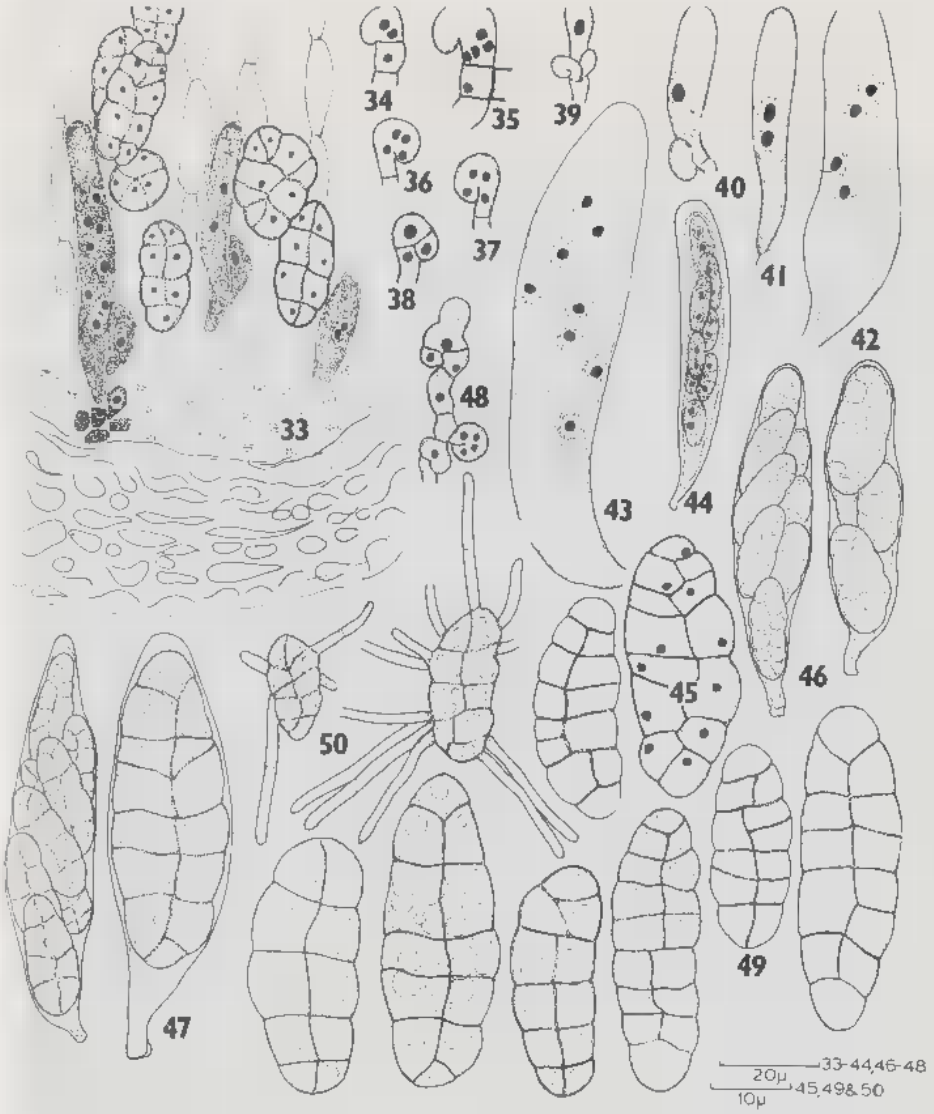
The first stage in the development of the perithecium is the development of the stroma. A highly branched and compact system of hyphae is produced and forms a plectenchymatous stroma. In due course the plectenchymatous stroma becomes pseudoparenchymatous. As already described, mature stroma (Plate I, f, g) is composed of thick-walled, pigmented cells towards the periphery and thin-walled hyaline cells in the interior.

The first indication of perithecial development is the formation of a deeply staining ascogonium embedded in the stroma, beneath the layers of pigmented, thick-walled cells (Fig. 18, 19; Plate I, g). When young, the ascogonium is composed of a globose, uninucleate cell and 1-2 septate, elongate, narrow trichogyne (Fig. 20; Plate I, h). The trichogyne disintegrates early. As the ascogonium makes its appearance, the surrounding stromal cells loose their identity. In later development, the ascogonium becomes a coiled structure with variable number (usually 7-8) of uni- or binucleate cells (Fig. 21, 22; Plate I, i). At this stage, the ascogonium is seen surrounded by 2-3 layers of polygonal cells which stain deeply compared to the cells of the stroma forming as it were an envelope. The origin of these cells is not clear. But from the fact that they take stain deeply and they are thin-walled shows that they are newly formed cells, almost at the same time as the development of the ascogonium. With further development, the cells constituting the envelope divide and thereby increasing in thickness of the envelope and in fact, this envelope of cells constitute then what becomes the wall of the perithecium. The periphery of the wall is confluent with the stroma (Fig. 23, 24). Three to four layers of the wall cells in the periphery become thick-walled and 2-3 layers of cells in the inner region remain thin-walled.

In the next stage, the perithecium is seen to protrude out of the stroma and is visible under the stereomicroscope as a tiny dot on the stromal surface. At this time, the centrum of the young perithecium becomes a site of great activity. Some of the cells immediately surrounding the ascogonium disintegrate and

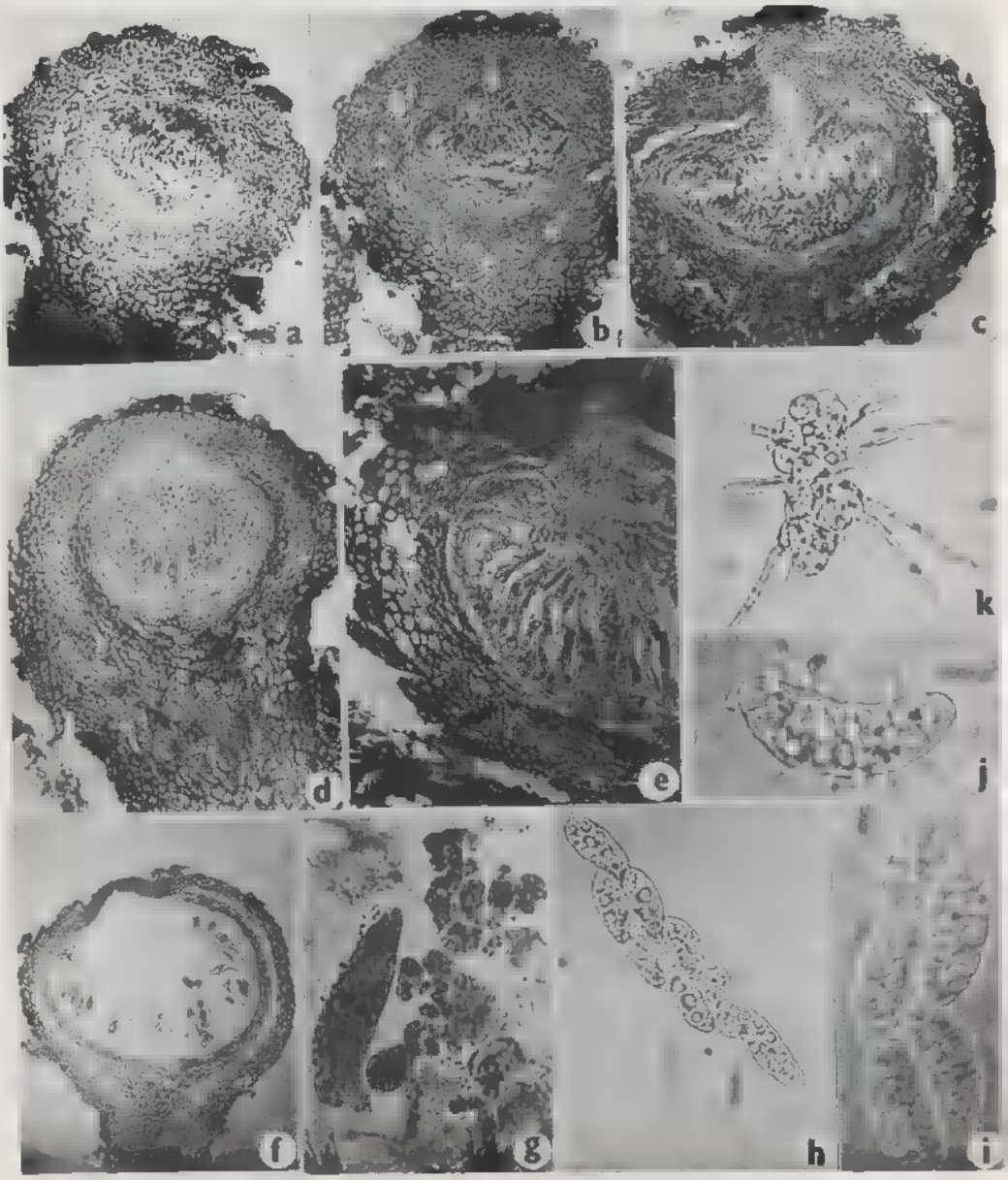

Figs. 29 - 32. - *Thyronectria pseudotrichia*
29 - 32 - further stages in the development of perithecium. Note the apical paraphyses in 29 and their gradual disappearance in 31 and 32.

Fig. 29 - 32. - *Thyronectria pseudotrichia*
29 - 32 - stades successifs du développement d'un périthèce. On notera la présence des paraphyses en 29 et leur disparition progressive en 31 et 32.




Figs. 33 - 50. – *Thyronectria pseudotrichia*
33 - portion of a mature perithecial centrum (enlarged); 34-44 - stages in the development of ascus and ascospores; 45-47 - mature asci; 48-49 - ascospores. Nota in 48, uninucleate cells in ascospore; 50 - germinating ascospores.

Fig. 33 - 50. – *Thyronectria pseudotrichia*
33 - région centrale d'un périthèce mûr; 34-44 - stades successifs de développement des asques et des ascospores; 45-47 - asques mûrs; 48-49 - ascospores; 50 - ascospores germant.



form a cavity around the ascogonium (Plate I, j). Cells of the enveloppe at the base of the cavity now enlarge in size. At the same time, cells of the envelope at the apex of the cavity divide repeatedly and form a zone of intensively staining, small, uninucleate cells which occupy a area below the morphological apex of the young perithecium (Fig. 24; Plate I, k). Curiously enough, this 'meristematic' zone at the apex becomes active and produces a palisade of downwardly growing septate filaments, the apical paraphyses, the cells of which are uninucleate (Fig. 25; Plate II, a, b). As the apical paraphyses grow downward, the young perithecium increases in size and the downwardly growing apical paraphyses keep pace with the expansion of the perithecium and fill the entire centrum cavity (Plate II, d). The centrally located ascogonial cells are pushed to the bottom of the cavity by the descending apical paraphyses (Fig. 26).

The globose cells at the bottom of the centrum cavity already referred to now become highly vacuolated and start desintegrating. The asci develop interspersed with apical paraphyses forming a kind of hymenium at the base and to some extent on the sides of the centrum (Fig. 27-29; Plate II, c, e).

At about the time the apical paraphyses touch the bottom of the cavity, there is an upward growth of the 'meristem' at the morphological apex of the perithecium (Fig. 26-28; Plate II, b, c). They grow rapidly and as these cells grow from all sides an ostiolar canal is formed schizogenously (Fig. 30). The cells constituting the ostiolar canal are compactly arranged and are in the form of unbranched hyphae.

In the final stages of the development of the perithecium, the thin-walled cells comprising the perithecial wall are separated from the surrounding thick-walled cells of the wall all along their length except at the bottom (Fig. 29-31; Plate II, f). This separation of the perithecial wall is apparently due to lysis of cells in that region.

## DEVELOPMENT OF ASCI

The ascogonial cells divide and produce a basal layer of variable number of ultimately binucleate cells. Ascogenous hyphae develop from the ascogonial cells and two nuclei migrate into each ascogenous hypha (Fig. 34). The binucleate ascogenous hypha elongates and becomes curved at the tip (crozier)

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Plate II. — *Thyronectria pseudotrichia*
■ - ■ - stages in the development of perithecial centrum and ostiole (note asci developing among apical paraphyses in e) x 210; f - section through a mature perithecium x 210; g - ascogenous system showing croziers x 630; h - an ascus x 630; i - ascospores ■ 630; j - an ascospore and ascoconidia x 630; k - germinating ascospore x 630.

Planche II. — *Thyronectria pseudotrichia*
a - ■ - différentes étapes du développement du centre du périthèce et de l'ostiole x 210; f - section dans un périthèce mûr x 210; ■ - système ascogène avec des croziers ■ 630; h - asque x 630; i - ascospores x 630; j - ascospore et ascoconidies x 630; k - ascospore germant x 630.

while the 2 nuclei divide simultaneously and become four (Fig. 35-57). Two septa divide the 4-nucleate crozier into a basal unicleate and an apical uninucleate cells in between separating a binucleate median cell. The two nuclei in the medium cell fuse (Fig. 38) and, further, the median cell elongates with the fusion nucleus migrating to midway position (Fig. 40). Meiosis occurs at this stage in the young ascus, first, becoming a binucleate ascus (Fig. 41), then a 4-nucleate ascus (Fig. 42). A third, mitotic, division is soon golowed resulting in an 8-nucleate ascus (Fig. 43). This is followed by another mitotic division resulting in an 16-nucleate stage. Cleavage furrows are laid down around each pair of nuclei in the young ascus delimiting eight young elliptical ascospores (Fig. 44). Spores are initially transversely septate, and later become muriform (Fig. 45).

By repeated crozier formation, each ascogenous hyphae may give rise to several asci (Fig. 48; Plate II, g).

## DISCUSSION

The anamorph of *Thyronectria pseudotrichia* is a synnematous, phialidic hyphomycete with slimy conidia assigned to *Stilbella cinnabarina*. The ascogonium, to begin with, is globose to subglobose, uninucleate, provided with a trichogyne, and develops in a pre-formed pseudoparenchymatous stroma. The ascogonium eventually becomes a several-celled, coiled structure. the cells being multinucleate, and the trichogyne disappears. Asci are produced by the intervention of croziers, the ascogenous hyphae being short. The asci are without any apical apparatus. The perithecial centrum is of the typical *Nectria*-type (LUTTRELL, 1951) with apical paraphyses. Thus *Thyronectria pseudotrichia* is a good hypocreaceous fungus. However, we cannot be certain that it is a *Thyronectria* as we have no precise, understanding of *T. patavina* Sacc. which is the type species of the genus. The type material has not been available to us for study and even SEELER (1940) who monographed the genus had to depend entirely on Saccardo's original diagnosis and illustrations in his circumscription of the genus.

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